Taxonomic status and phylogenetic relationship of some Algerian shallow-water sea cucumber species (Holothuroidea: Echinodermata) deduced from mitochondrial DNA sequences

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Abstract—The present study focuses on the taxonomic revision and phylogeny of some shallow-water Algerian sea cucumber species belonging to the genus *Holothuria* (the two morphotypes (A & B) of *Holothuria* (Holothuria) *tubulosa* (Gmelin, 1791); *Holothuria* (Roweothuria) *tubulosa* (Delle Chiaje, 1824) and *Holothuria* (Roweothuria) *arginensis* (Koehler et Vaney, 1906)). The nucleotide sequences of the 16S rRNA gene obtained by [1] were used to construct the phylogenetic tree of the studied specimens. Our results showed that the two morphotypes (A & B) of *Holothuria (H.) tubulosa* are genetically separated. We also reported a close genetic relationship between the two morphotypes of *Holothuria (H.) tubulosa* (A & B) and *Holothuria (R.) arguinensis*. *Holothuria (R.) poli* is the most divergent species and is in a paraphyletic position with the other studied specimens. Sequence analyses of the 16S rRNA gene revealed the presence of 58 polymorphic sites: 79.25% of these sites are parsimoniously informative and 20.75% are singletons. The means of haplotype diversity (Hd) and nucleotide diversity (π) were very high.

Keywords—Echinodermata, Holothuria, Phylogenetic systematics, 16S rRNA, Haplotypes, Algerian basin.

INTRODUCTION

Aspidochirotid holothurians, commonly known as "sea cucumbers", are marine invertebrates belonging to the echinoderm group. There are over 1400 species occurring from the intertidal to the deepest oceanic trenches [2]. Holothurians are among the main sediment bioturbators in marine ecosystems [3]. They ingest the soft substratum and remove the organic matter which is necessary for their growth. This process prevents the accumulation of organic matter in the marine sediment and could help to control the effect of pathogenic agents [4]. The ecological role of the holothurians species is largely well known by scientists, but there is a lack of informations concerning the taxonomy of several groups of species belonging to the genus *Holothuria* [5]. In fact, this genus contains individuals with few taxonomically informative morphological characteristics and with a high degree of intraspecific phenotypic diversity [6].

MATERIAL AND METHODS

The present study is a part of a further systematic review for data already obtained by [1] on the phylogeny of some aspidochirotid holothurians species of the Algerian coast. Two Mediterranean species [Holothuria (Holothuria) *tubulosa*; *Holothuria* (Roweothuria) *poli* and one exotic species originating from Atlantic Ocean [Holothuria (Roweothuria) *arginensis* have been considered. Most "sea cucumbers" individuals were collected from three stations located in the centre of the Algerian coast [Sidi-Fredj (2° 50.55'E-36° 45.45'N), Tamentefoust (3° 14.00'E-36° 48.33'N) and Figuier-plage (3° 30.92'E-36° 47.05'N)]. Sampling was also carried out in two other stations, the first located in the western part of the Algerian coast [Stidia (0° 1.11’O-35° 49.85’N)] and the second in the French coast [Banyuls sur mer (3° 7.87’E-42° 28.95’N)]. The samples were collected by scuba diving between 0.5 and 10m depth [1].The sequences data obtained from the portion of mitochondrial gene16S rRNA by [1] were classified on the software "BioEdit" [7] and aligned using the extension "CLUSTAL W" integrated in "BioEdit" [8]. After alignment, the sequences were classified on the "Mega 5" software [9]. Relationships between individuals or populations are visualized through phylogenetic tree using the Maximum
Likelihood method [10]. We used the software "jModeltest2.1.3" to find the best model of nucleotide substitutions. The Kimura model with 2 parameters (K2P + gamma distribution) was the most adequate. For evaluating the robustness of the root nodes of the reconstituted tree, we performed 100 Bootstrap replicas. The sequence of Holothuria (Platyperona) forskali obtained from "Genbank" was used as an out group. Sequencing of a 411Pb fragment along the mitochondrial rRNA16S gene of the studied species revealed the existence of 58 polymorphic sites; 79.25% of these sites are parsimoniously informative and 20.75% are singletons. For this, the haplotype diversity (Hd), nucleotide diversity (π) and McDonald's-Kreitman test [11] were estimated by the DNASP 5.10 program [12].

RESULTS

The obtained phylogenetic tree present four clades with high Bootstrap values (Fig. 1). The two morphotypes of Holothuria (H.) tubulosa (A & B) are genetically separated. Holothuria (R.) arguinensis is much closer to H. (H.) tubulosa (morphotype A) than H. (R.) poli (Fig. 1). Both varieties of H. (H.) tubulosa (A & B) and H. (R.) arguinensis are related and monophyletic species. Holothuria (R.) poli appears to be the most divergent and distant species (Fig. 1). The means values of haplotype diversity (Hd) and nucleotide diversity (π) are presented in Table I. The results of McDonald's-Kreitman's test are summarized in Table II.

![Figure 1: Phylogenetic tree established for the studied aspidochirotid holothurians species by the Maximum likelihood method (HaA: H. (H.) tubulosa A; HaB: H. (H.) tubulosa B; Ha: H. (R.) arguinensis; Hp: H. (R.) poli; S: Stidia; F: Figuer-Plaże; T: Tamentefoust; BM: Banyul-sur-Mer) (100 Bootstrap replicas).](image)

Table I: Genetic diversity of the studied aspidochirotid holothurians species. NS: number of sequences, h: number of haplotype, Hd: haplotype diversity, π: nucleotide diversity.

<table>
<thead>
<tr>
<th>Species</th>
<th>NS</th>
<th>h</th>
<th>Hd</th>
<th>π</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. (R.) poli</td>
<td>18</td>
<td>4</td>
<td>0.82</td>
<td>0.004</td>
</tr>
<tr>
<td>H. (H.) tubulosa A</td>
<td>8</td>
<td>4</td>
<td>0.64</td>
<td>0.004</td>
</tr>
<tr>
<td>H. (H.) tubulosa B</td>
<td>6</td>
<td>4</td>
<td>0.80</td>
<td>0.005</td>
</tr>
<tr>
<td>H. (R.) arguinensis</td>
<td>3</td>
<td>3</td>
<td>1.00</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table II: The McDonald-Kreitman (1991) test between the studied holothurians species.

<table>
<thead>
<tr>
<th>Species</th>
<th>KN/KS</th>
<th>ON/OS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. (R.) poli and H. (R.) arguinensis</td>
<td>3.16</td>
<td>2.00</td>
<td>0.69</td>
</tr>
<tr>
<td>H. (R.) poli and H. (H.) tubulosa A</td>
<td>2.30</td>
<td>2.50</td>
<td>1.00</td>
</tr>
<tr>
<td>H. (R.) poli and H. (H.) tubulosa B</td>
<td>2.50</td>
<td>3.33</td>
<td>0.70</td>
</tr>
<tr>
<td>H. (R.) arguinensis and H. (H.) tubulosa A</td>
<td>2.33</td>
<td>1.00</td>
<td>0.63</td>
</tr>
<tr>
<td>H. (R.) arguinensis and H. (H.) tubulosa B</td>
<td>0.62</td>
<td>1.33</td>
<td>0.64</td>
</tr>
<tr>
<td>H. (H.) tubulosa B and H. (H.) tubulosa A</td>
<td>1.37</td>
<td>2.00</td>
<td>0.70</td>
</tr>
</tbody>
</table>

DISCUSSION

Along the history of the classification of the aspidochirotid holothurians species, taxonomists were sure and all agreed that Holothuria (H.) tubulosa, the best-known and the most common species in the Mediterranean Sea, presents only one well separated species. The obtained phylogenetic tree indicates that Holothuria (H.) tubulosa is represented by two varieties of species: The first is named in the present work classical Holothuria (H.) tubulosa A, because of its morphological characteristics which are very similar to those already described by [13] and the second named in the present work Holothuria (H.) tubulosa B, which has different morphological characteristics and could be considered as a new to science. According to the obtained results, these two species varieties are genetically distinct (Fig. 1). This result has already been reported by [1] and [14]. These differences do not concern only the obtained molecular data, but also the anatomical, morphological and endoskeletal dat [1-15]. Holothuria (R.) arguinensis is much closer to both populations of H. (H.) tubulosa (A & B) than to Holothuria (R.) poli (Fig. 1). Reference [16] reported a very close relationship between H. (H.) tubulosa and H. (R.) arguinensis and this is the case in this study. Holothuria (R.) arguinensis is an Atlantic exotica species not endemic to the Mediterranean Sea. This species is reported for the first time in the Algerian coast by [17]. The phylogram (Fig. 1) also illustrates that Holothuria (R.) poli is the most divergent species. Our results are in contradiction with previous works, which are based only on the endoskeletal criterion. More precisely, the Holothuria and Roweothuria subgenera do not appear as reciprocally monophyletic groups [18-19]. According to our results both subgenus are paraphyletic. The phylogenetic tree gives us an indication of
the subgenus Platyperona (Fig. 1). This subgenus is among the oldest and most divergent lineages in the genus Holothuria [16], which is in agreement with the work of [20]. This justifies it choice as an external group. Their basal position on the phylogenetic tree (Fig. 1) is in agreement with the plesiomorphic calcareous rings found in individuals of this genus [16]. Calcareous rings are very similar to those observed in the fossils reported by [21]. This supports the idea of treating it as genera and not as subgenera. The low nucleotide diversity (π) reflects the presence of a dominant haplotype and many related haplotypes, which explains the high value of haplotypic diversity (Hd) (Table I). Concerning the McDonald's-Kretman's test, the p-values were not significant (Table II). We noted a Darwinian selection between taxa, more specifically between H. (R.) poli and H (R.) arguinensis and between H. (H.) tubulosa A and H. (R.) arguinensis (Taba II). A retention of an ancestral polymorphism between taxa in the case where Kn/Ks<On/OS (Taba II).

CONCLUSION

The present study allowed to revaluate the taxonomic status of some aspidochirotid holothurians species of the Algerian coast. The obtained results indicate that Holothuria (H.) tubulosa, the most common species in the Mediterranean Sea, has two distinct species that have been noted in the present work as morphotype A & morphotype B. The morphotype "A" corresponds to the classical Holothuria (Holothuria) tubulosa previously described by [13]; while the morphotype "B" should corresponds to a new species whose molecular characteristics are different from those of Holothuria (H.) tubulosa A. We also reported a close relationship between Holothuria (H.) tubulosa A and Holothuria (R.) arguinensis which corroborates with other studies carried out on the genus Holothuria in the Mediterranean Sea and the Atlantic Ocean. The genetic tests showed very high haplotypic and nucleotide diversities.

References


